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# CORRELATION OF HYPOXIA INDUCIBLE FACTOR-1α LEVELS WITH DISEASE STATUS IN ORAL SQUAMOUS CELL CARCINOMA

Biochemistry

**KEYWORDS:** HIF-1 α, OSCC

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## ABSTRACT

Introduction: oral squamous cell carcinoma (OSCC) is a major health problem in our country. the increasing incidence of youth dependence on tobacco , pan masala and gutka, which are known pre disposing factors have further aggravated the problem. Intra tumoral hypoxia activates a number of adapting pathways such as the Hypoxia Inducible Factor-1α(HIF-1α) which confers a survival advantage to the tumour cells. The present study was carried out to evaluate the association of HIF-1 expression with disease aggressiveness.

Material & Methods: The study comprised of 50 histologically proven cases of oral SCC and 50 healthy controls. The HIF -1 α levels were measured by commercially available ELISA in the blood samples. The data was analysed using SPSS software version 20.

Results: The HIF-1α levels were found significantly higher in the patients with aggressive disease regarding the histological grading of differentiation and the stage at presentation.

Conclusions: Our findings prove that higher HIF-1α levels indicate advanced state of the disease and consequent poor prognosis.

**Introduction**

Epidemiologically, oral cancer is the third most common malignancy in India[1]. Approximately 90% of oral cancers are squamous cell carcinomas [2, 3]. The frequency of oral cancer is high in India because of cultural, ethnic, geographic factors and the popularity of addictive habits, it ranks number one in terms of incidence among men and third among women. Cancer of buccal mucosa, lower alveolus and the retro molar trigone are grouped together as cancers of gingivo-buccal complex and can be called as the "Indian Oral Cancer"as they constitute 60% of all oral cancers in India [4].

The increasing incidence of alcoholism, smoking and consumption of tobacco, pan masala and paan (betel quid) have led to the geometric increase in incidence. The initiation of these habits at an early age further aggravates the problem. The lack of oral hygiene and awareness about pre-malignant conditions lead to detection at a later stage. It has one of the lowest 5-years survival rates of all cancers, probably because most lesions are missed in the initial stages[5] .The detection at later stages followed by high incidence of recurrence makes oral cancer a serious health problem in our country.

In state of tumor growth, cancer cells continue to proliferate near supplying blood vessel and ultimately massive growth of the tumor renders its distant cells in a state of hypoxia. Mammalian cells have developed a range of adaptations to survive acute and even prolonged hypoxia. This activates the hypoxia inducible factor-1 (HIF-1) signaling cascade. Hypoxia-inducible factor 1 (HIF-1) is a transcriptional activator that functions as a critical regulator of oxygen homeostasis [6]. It is a heterodimer composed of two subunits, HIF-1α and HIF-1β, which dimerize and bind to DNA via the basic helix-loop-helix domain [7, 8] . HIF-1 α can activate the transcription of more than 60 target genes that are involved in crucial aspects of cancer establishment, including cell survival, glucose metabolism, angiogenesis and invasion [9, 10]. The synergistic role of tobacco and arecoline in HIF-1 α expression has also been reported.

The present study was conducted to evaluate the association between disease progression and HIF-1αlevels in oral squamous cell carcinoma.

**Material and methods**

The study was conducted in the Department of Biochemistry in collaboration with the Department of Dental and OromaxillofacialSurgery, Lady Hardinge Medical College & Smt. Sucheta Kriplani Hospital, New Delhi. The study was commenced after prior approval from the institutional ethical committee. Fifty histologically confirmed cases of squamous cell oral carcinoma attending the outpatient and inpatient department of Dental and Oro-maxillofacial Surgery in Smt. Sucheta Kriplani Hospital , LHMC, New Delhi were enrolled as cases during the study period. The controls were recruited from the departments of Dental and Oromaxillofacial Surgery and Biochemistry fulfilling inclusion criteria.

*Inclusion criteria*

Cases: 50 Patients with histologically confirmed oral squamous cell carcinoma.

Controls: 50 healthy age and sex matched healthy volunteers from the department of Biochemistry and Dental and Oromaxillofacial Surgery fulfilling the following criteria.

1. No past history of any malignancy

2. Apparently healthy oral healthy cavity on inspection.
3. No history of acute / chronic illness

*Exclusion criteria*
Case
1. Past history of any ischemic disease ex: cerebral ischemia, myocardial infarction etc
2. Initiation of chemotherapy or radiotherapy for oral cancer

*Sample collection and analysis*
Ten ml of venous blood sample was collected from the subjects under sterile conditions. The blood samples were processed immediately for separation of serum which was subsequently aliquoted and stored at - 40ºC for further analysis. Repeated freeze-thaw cycles were avoided.

All routine blood investigations were carried out on fully automated analysers using standard reagents. HIF-1αα levels were estimated by commercially available ELISA kit supplied by QayeeBio(China).

*Statistical methods*
All analyses were performed with the SPSS software programme version 20. For comparison of variables with a normal distribution unpaired, 2-tailed Student's t-test and Pearson's correlation were used. A p≤0.05 was considered statistically significant. Logistic regression analysis was used to identify association with risk factors.

**Results**
Table no 1 depicts the demographic characteristics of the study population. The cases and controls were age and sex matched. A high incidence of the risk factors for oral cancer (intake of alcohol, khaini, tobacco, sharp tooth, regurgitation of food) is seen in the cases with oral cancer.

Table nos 2& 3 summarize the stage and histological grade of oral squamous cell carcinoma in the cases. Majority of the cases presented in stage III (43/50). The tumours were moderately differentiated in approximately 50% of the cases.

Figure no 1 is the representation of the HIF-1A alpha levels in the cases as compare to controls. The levels were significantly higher in the cases as compared to the controls

**Discussion**
Cancer is a multifactorial disease which results from a complex interplay between environmental and genetic factors. It has become one of the most challenging health issues today11. Intratumoral hypoxia is a common feature in solid tumours. Hypoxia inducible factor 1 alpha (HIF-1Aα) is a transcription factor that initiates the expression of genes involved in neo-vascularization and glucose metabolism that confers survival advantage.

We enrolled 100 subjects in the study after prior informed and written consent. The study group consisted of 50 patients of biopsy proven cases of oral squamous cell carcinoma, presenting to dental &oro-maxillofacial surgery department, along with 50 age and sex matched healthy volunteers as controls. The study group constituted of predominantly males (82%) with the mean age being approximately 50 years.

In our study 86% of the cases presented in stage 3 to the OPD for evaluation while 46% of the patients had histologically well differentiated tumours at the time of presentation. In a study conducted by Gorsky etal12 half of the cases were diagnosed at advanced stage, while Chan et al13reported 72% in with stage I, 38.9% in stage II, 26.7% with stage III, and 11.8% with stage IV cancer. In our study, we found that the out of 50 cases, 10(20%) were well differentiated, 26(46%) moderately differentiated and 14(28%) were poorly differentiated. Our findings are in accordance with a similar study carried out by Udeabor et al14 who reported 18% as well differentiated,63.2% asmoderately differentiated and 13.2% poorly differentiated.

The HIF-1αα levels were found to be 767± 283.2 pg/ml and 417 ±145.5pg/ml in cases and controls respectively. This difference was found to be statistically significant with p-value of <0.001 which is comparable to study done by Jia et al15 . It could possibly be due to the increased demand of oxygen by cancer cells. HIF-1 α is a key transcription factor that regulates cellular reaction to hypoxia and is over expressed in most solid tumors, in response to low oxygen concentrations . It has the ability to influence metabolic reprogramming, angiogenesis, cell survival, and energy metabolism.

Zhu et al 16 also reported that HIF1α level was significantly associated with T stage, lymph node involvement, histologic differentiation and microvessel density. Patients with positive HIF1α nuclear staining had a significantly worse overall survival and disease-free survival. Kang et al17also found that HIF1α over expression was significantly associated with poor overall and disease-free survival rates, independent of T stage and lymphatic metastasis. The Cox proportional hazards regression model demonstrated that the level of HIF1α expression may be an independent prognostic factor for tongue SCC. Eckert et al18 demonstrated that increased HIF1α expression, alone or in combination with a low CAIX(carbonic anhydrase IX) expression, was significantly correlated with a poor prognosis of OSCC.

These studies in congruence with our finding prove that higher HIF-1α levels indicate advanced disease status and call for aggressive management and frequent follow up visits. This also re-emphasizes the need for individualized anti- HIF therapy in such patients.

**ACKNOWLWDGEMENTS: None**

**Table no 1: DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION**

| | **Cases (n=50)** | **Controls (n=50)** |
|---|---|---|
| Age (Yrs) (Mean ±SD) | 50.32±8.04 | 50.15±8.03 |
| Sex M/F | 41/9 | 41/9 |
| Tobacco Chewing | 40 | 3 |
| Smoking | 30 | 12 |
| Alcohol | 24 | 10 |
| Khani | 11 | 3 |
| H/O Hypertension | 9 | 2 |
| H/O Diabetes Mellitus | 1 | 0 |
| H/O Hot Spicy Food | 8 | 5 |
| H/O Sharp Tooth | 3 | 0 |
| H/O Regurgitation of food | 12 | 5 |
| family h/o cancer | 1 | 0 |

**Table no 2: STAGE OF ORAL SQUAMOUS CELL CARCINOMA OF THE CASES AT THE TIME OF PRESENTATION WITH** hif **LEVELS**

| **Stage** | **Cases (n=50)** | **Percentage (%)** | **HIF LEVELS** |
|---|---|---|---|
| I | Nil | Nil | Nil |
| II | 4 | 8 | 688±163 |
| III | 43 | 86 | 768±157 |
| IV | 3 | 6 | 853±170 |

**Table no 3: HISTOLOGICAL GRADE OF ORAL SQUAMOUS CELL CARCINOMA OF THE CASES AT THE TIME OF PRESENTATION**

| **Histopathological grading** | **Cases (n=50)** | **%** | **HIF LEVELS** |
|---|---|---|---|
| Well differentiated | 10 | 20 | 536±185 |
| Moderate differentiated | 26 | 52 | 724±208 |
| Poor differentiated | 14 | 28 | 1078±210 |

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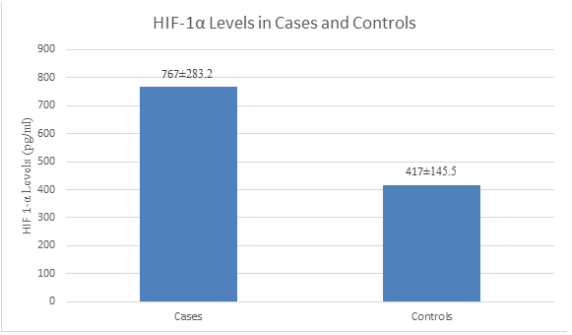
**FIGURE LEGENDS: HIF-1 α levels in the cases and controls**

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